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A specific transcriptional response of yeast cells to camptothecin dependent on the Swi4 and Mbp1 factors

Luca Lotito [a], Alessandra Russo [a], Susana Bueno [b], Giovanni Chillemi [b], Maria Vittoria Fogli [a], Giovanni Capranico [a,*]

[a] "G. Moruzzi" Department of Biochemistry, University of Bologna, via Irnerio 48, 40126 Bologna, Italy
[b] CASPUR, Rome, Italy

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ABSTRACT

Topoisomerase I (Top1) is the specific target of the anticancer drug camptothecin (CPT) that interferes with enzyme activity promoting Top1-mediated DNA breaks and inhibition of DNA and RNA synthesis. To define the specific transcriptional response to CPT, we have determined the CPT-altered transcription profiles in yeast by using a relatively low concentration of the drug. CPT could alter global expression profiles only if a catalytically active Top1p was expressed in the cell, demonstrating that drug interference with Top1 was the sole trigger of the response. A total of 95 genes showed a statistically-significant alterations. Gene Ontology term analyses suggested that the cell response was mainly to the inhibition of nucleic acid synthesis and cell cycle progression. Promoter sequence analyses of the 22 up-regulated genes and expression studies in gene-deleted strains showed that the transcription factors, Swi4p and Mbp1p, mediate at least partially the transcriptional response to CPT. The *MBP1* gene deletion abrogates a transient cell growth delay caused by CPT whereas the *SWI4* gene deletion increases yeast resistance to CPT. Thus, the findings show that yeast cells have a highly selective and sensitive transcriptional response to CPT depending on *SWI4* and *MBP1* genes suggesting a complex regulation of cell cycle progression by the two factors in the presence of CPT.

1. Introduction

The selective target of camptothecin (CPT), an effective antitumor agent, is DNA topoisomerase I (Top1), a nuclear enzyme that regulates DNA superhelicity during fundamental processes such as DNA replication, recombination and repair, transcription, chromatin remodeling and chromosome condensation and segregation (Wang, 2002). Top1 is required for the development of higher eukaryotes, however the *TOP1* gene is not essential in *S. cerevisiae* likely because DNA topoisomerase II can substitute for Top1 (Champoux, 2001; Wang, 2002). During the enzyme catalytic cycle, a Tyr residue cleaves a strand of a DNA duplex forming a covalent bond with its 3' end, and after the rotation of the free 5' end around the uncut strand, Top1 restores the intact duplex (Champoux, 2001). CPT binds noncovalently to a transient DNA-enzyme complex reducing the DNA religation rate of the enzyme (Capranico et al., 1997; Li and Liu, 2001; Pommier, 2006; Staker et al., 2002). As a consequence, Top1-linked DNA single-stranded breaks accumulate in the cell, rapidly resulting in a marked inhibition of DNA and RNA synthesis (Li and Liu, 2001; Pommier, 2006). However, CPT-promoted DNA lesions are highly reversible both *in vitro* and *in vivo*, and cell lethality can thus occur when the cleavable complex is transformed into a double strand DNA break by the progression of replication forks or other nuclear processes (Li and Liu, 2001; Pommier, 2006).

In yeast, genome-wide phenotyping studies aimed at identifying genes involved in cellular recovery following treatments with DNA-damaging agents, such as methyl methane sulfonate (MMS) (Jelinsky et al., 2000), suggest that many diverse molecular processes are needed for the recovery of *S. cerevisiae* (Fry et al., 2005; Jelinsky et al., 2000). In particular, more than one third of ~6200 *S. cerevisiae* genes respond to the exposure to at least one of several DNA-damaging agents tested in a time- and dose-dependent manner (Jelinsky et al., 2000). Recent investigations have emphasized the complexity of the yeast response to DNA damage (Cagney et al., 2006; Fry et al., 2005), in agreement with the early observation of a general stress response pathway (Environmental Stress Response) that is activated by different environmental changes potentially harmful to yeast cells (Cagney et al., 2006; Gasch et al., 2000, 2001). Interestingly, little relationship exists between genes activated following UV radiation, ionizing radiation, cisplatin and oxidative stress and genes proven to confer resistance/tolerance to the studied agents (Begley et al., 2002; Birrell et al., 2002; Fry et al., 2005). It has been suggested that genes showing an alteration of the drug sensitivity phenotype were more likely to function upstream in a pathway, whereas genes showing transcriptional response were more likely to encode proteins functioning downstream (Fry et al., 2005). However, as commonly-studied agents damage proteins, RNA and lipids in addition to DNA,

* Corresponding author. Tel.: +39 051 2094282; fax: +39 051 2094283.
E-mail address: giovanni.capranico@unibo.it (G. Capranico).

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the cell response to them also includes activation of pathways of protein and lipid turnover (Fry et al., 2005). That explains, at least in part, the vast transcriptional response to damaging agents such as alkylating agents, oxidative stress and others. Strikingly, it has not yet been established the yeast response to a compound that specifically damages the DNA. In particular, the specific transcriptional response to CPT has not yet been established at a global level.

Thus, we have here asked whether a specific response to CPT inhibition of Top1 can be identified in yeast. To this end, we have determined the expression profiles following CPT treatments in yeast cells expressing a wild type or an inactive mutant yeast Top1. The findings show that yeast cells have a specific transcriptional response to CPT, which, while transiently decreasing the cell growth rate, triggers a new balance of global transcript levels depending, at least partially, on the Mbp1/Swi4 gene regulatory network that may set a proper progression of the cell cycle in the presence of CPT.

2. Materials and methods

2.1. Yeast strains and plasmids

The studied *S. cerevisiae* strains are: JEL1Δ*top1* (*MAT*α, *leu2*, *trp1*, *ura3-52*, *prb1-1122*, *pep4-3*, *his3*Δ::*PGAL1-GAL4*, *top1Δ*) (Scaldaferro et al., 2001), LUC1 (*swi4*Δ*::kanMX4* in the JEL1Δtop1 background), DAV1 (*mbp1*Δ*::kanMX4* in the JEL1Δtop1 background), MAV1 (*mbp1*Δ*::kanMX4* in the JEL1Δtop1 background). The PCR-based gene deletion strategy (Wach et al., 1994) was used to replace by homologous recombination the whole *SWI4* and *MBP1* coding sequences in JEL1Δ*top1* yeast cells (Scaldaferro et al., 2001) with a KanMX cassette conferring geneticin resistance.

The plasmids used in this study are: pCC10 (a low copy-number plasmid), which expresses the yeast *TOP1* gene under its own promoter (Vogelauer and Camilloni, 1999); pAR7, a derivative of pCC10 expressing a Y727F mutant enzyme (Lotito et al., 2008); pCC10HA and pAR7HA, corresponding to pCC10 and pAR7, respectively, expressing the corresponding HA-tagged enzyme; pEZ2T-Top1, a high-copy number plasmid expressing a wild type human Top1 (Scaldaferro et al., 2001); YCp50, and YepSec as the empty vector control (Scaldaferro et al., 2001). The plasmid pBA417, carrying the yeast *SWI4* gene, was kindly provided by B. Andrews (University of Toronto, Toronto, Canada). The plasmid pFA6-kanMX4, kindly provided by P. Philippsen (Biozentrum der Universitat Basel, Basel, Switzerland), carries the geneticin-resistance gene. The plasmid pSL1, carrying the yeast *MBP1* gene, was constructed by inserting into the pPC86 (Invitrogen) plasmid the entire *MBP1* ORF under the *ADH1* gene promoter.

2.2. Cell treatments

Yeast cells were grown in SC-uracil medium supplied with 25 mM HEPES, pH = 7.0 and 2% glucose. A single colony of *S. cerevisiae* Jel1ΔTop1, carrying the pCC10 or pAR7 plasmids, was inoculated into 40 ml of SC-uracil medium for expression profiling experiments. Cells were then grown at 30 °C on a shaker (190 rpm) 24 hours, then, yeast cells were diluted to an OD_{600} = 0.02 and grown overnight. Cells were treated with 50 μM CPT, (S)-(+)-camptothecin (Sigma), or 0.05% dimethyl sulfoxide (Sigma) as control sample. Cell cultures were harvested after 5 hours of drug treatment. Cell pellets were then stored at −80 °C until RNA extraction. We prepared two independent cultures for each sample, and the corresponding total RNAs were pooled together.

2.3. Microarray hybridizations, data normalization and statistical analyses

The complete procedures were described elsewhere (Lotito et al., 2008). Briefly, for each sample, the cDNA was prepared from the poly $(A)^+$ RNA fraction purified from a pool of two independent cell cultures. cDNAs were then fluorescently labelled and hybridized to cDNA slides containing 6155 verified yeast ORFs and prepared as described previously (Cavalieri et al., 2000; Schaus et al., 2001). Each experiment was repeated twice. Fluorescence data have been normalized by applying the print tip group normalization (Lotito et al., 2008). Full datasets were deposited into ArrayExpress under the accession number E-MEXP-1104.

2.4. Transcript level measurements by quantitative real-time (qrt)-PCR

cDNAs were prepared from total RNA (50 μg) isolated from control or CPT-treated yeast populations by using a reverse transcriptase (Superscript II, Invitrogen) as described (Lotito et al., 2008) (see also relevant information at ArrayExpress, accession number E-MEXP-1104). The *TOP1* gene deletion and transcript levels have been assessed with RT-PCR and primers Leoyea1 and Leoyea2 (see Supplementary information). cDNA was retrotrascribed from total RNAs extracted from yeast cells expressing the plasmid-borne Top1p or the Top1-Y727F mutant. Qrt-PCR determinations were performed with a LightCycler (Roche) and FAST Start DNA Master SYBR Green I kit (Roche). Genomic DNA was used as standard to quantify samples. PCR reactions were performed with 0.35 μM each primer, 0.10 mM Master Mix (Roche), 3 mM $MgCl_2$ in 12 μL reaction volume. Primer sequences can be found in Table 1S of Supplementary information.

2.5. MEME analyses of promoter sequences

We searched for common motifs in the promoter sequences of up- and down-regulated genes with the free MEME software at the link http://meme.sdsc.edu/meme/intro.html. We used 500 bp upstream from the ATG of the inquired genes to perform the analyses. We searched for common motifs that are distributed among the sequences with any number of repetitions. MEME finds the optimum width of each motif within the limits 5 and 20 base pairs. We select to find a maximum of 6 common motifs. As control, we performed identical searches with shuffled letters as input sequences, which found no common motifs.

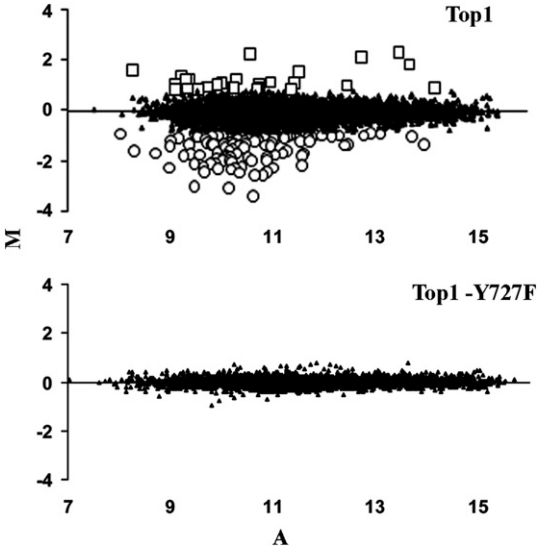

Fig. 1. Selective modifications of global expression profiles in *S. cerevisiae* by a minimally-cytotoxic CPT concentration (50 μM). M/A plots of fluorescence intensities for CPT-treated versus untreated cells expressing wild type Top1p (Top1) or the inactive Y727F mutant Top1p (Top1-Y727F). The plots show the log-ratio of the two expression intensities versus the mean log-expression of the two. Each symbol corresponds to a gene, and squares and circles are up- and down-regulated genes, respectively. A representative experiment is shown. Therefore, the numbers of altered genes is different from Table 1, as the Table lists genes that were significantly altered in two different experiments.